

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claims 1-30. (cancelled)

Claim 31. (currently amended): A method for quantifying an analyte in a specimen, said method comprising the steps of:

a) combining said specimen with an internal references species (IRS) of known concentration, in order to calibrate all subsequent steps; whereby said combination is referred to as an IRS-containing specimen;

b) combining said IRS-containing specimen with an affinity reagent, capturing and isolating said analyte and said IRS, wherein said IRS is a modified analyte with shifted molecular weight which binds to said affinity reagent;

c) analyzing and quantifying said analyte wherein analyzing and quantifying comprises using ~~only mass spectrometric analysis~~ matrix-assisted laser desorption/ionization (MALDI) to resolve distinct signals for said analyte and said IRS to determine the ratio of the analyte signal to the IRS signal.

Claim 32. (withdrawn): A method according to claim 31, in which said quantifying step further comprises using standard addition analysis.

Claim 33. (previously presented): A method according to claim 31, in which said quantifying step further comprises working curve analysis.

Claim 34. (withdrawn): A method according to claim 31, in which said quantifying step further comprises using working curve analysis calibrated only at a single point.

Claim 35. (withdrawn): A method according to claim 31, in which said quantifying step further comprises using multiple internal reference species at varying concentrations to display a working curve directly in a single mass spectrum which also contains the analyte signal.

Claim 36. (withdrawn): A method according to claim 32, in which said standard addition analysis comprises substeps of first, dividing said IRS-containing specimen into at least two IRS-containing sub-specimens, a first one of which being designated as an addition-free IRS-containing sub-specimen; obtaining an addition-free mass

spectrum of said addition-free IRS-containing sub-specimen; adding a known amount of said analyte (or counterpart thereof) to at least one of the remaining of said IRS-containing sub-specimens to form at least one addition-present IRS-containing sub-specimen in which the concentration ratio of the analyte (or a counterpart thereof) to the IRS has been increased by a known amount; and then obtaining an addition-present mass spectrum of each of said addition-present IRS-containing sub-specimens; and using the ratio of the analyte signal (or the counterpart signal) to the IRS signal in said addition-free mass spectrum and each said addition-present mass spectrum to quantify said analyte.

Claim 37. (withdrawn): A method according to claim 36 in which said substep of using the ratio of the analyte signal (or a counterpart signal) to the IRS signal in said addition-free mass spectrum and each said addition-present mass spectrum to quantify said analyte comprises a substep of normalizing said addition-free mass spectrum and each said addition-present mass spectrum by dividing each mass spectrum by the respective IRS signals to determine the ratios of the analyte signals (or the counterpart signals) to the IRS signals for each mass spectrum.

Claim 38. (withdrawn): A method according to claim 36 in which said substep of obtaining an addition-present mass spectrum of an addition-present IRS-containing sub-specimen is repeated in a plurality of distinct, successive substeps to obtain a plurality of distinct, successive addition-present mass spectra of a plurality of distinct, successive addition-present sub-specimens of said IRS-containing specimen in which the concentration ratio of the analyte signal to the IRS signal is successively increased in known amounts.

Claim 39. (withdrawn): A method according to claim 38 in which said substep of adding a known amount of said analyte or a counterpart thereof to said IRS-containing specimen is repeated a plurality of successive times; whereby each repeated substep of adding a known amount of said analyte or a counterpart thereof precedes each of said distinct, successive substeps for obtaining each of said plurality of distinct, successive addition-present mass spectra.

Claim 40. (previously presented) A method according to claim 33, in which said working curve analysis comprises substeps of:

- a) making a plurality of standard preparations, each containing a known but differing concentration of the analyte and each containing a known concentration of IRS;
- b) obtaining respective mass spectra of each of the plurality of standard preparations;
- c) normalizing each of the mass spectra from the plurality of standard preparations by dividing each mass spectrum by the IRS signal within the mass spectrum;
- d) creating a working curve by equating the normalized analyte signals to the analyte concentration of the plurality of standard preparations;
- e) obtaining a mass spectrum for the IRS-containing specimen;
- f) normalizing the mass spectrum of the IRS-containing specimen by dividing by the IRS signal within the mass spectrum; and
- g) quantifying the concentration of the analyte in the specimen using the working curve.

Claim 41. (cancelled)

Claim 42. (withdrawn): A method according to claim 34, in which said working curve analysis comprises substeps of:

- a) obtaining a first mass spectrum of a first portion of said IRS-containing specimen; then
- b) making a single standard preparation containing a known amount of said analyte or a counterpart thereof and containing a known amount of said IRS which is known relative to the IRS concentration in said IRS-containing specimen; then, obtaining a mass spectrum of said standard preparation; whereby said mass spectrum of said standard preparation provides a single point working curve relationship of mass spectra relative to analyte concentration; and then,
- c) using said first mass spectrum and the standard preparation mass spectrum single point working curve relationship to quantify said analyte.

- Claim 43. (withdrawn): A method according to claim 42 in which said substep of using said first mass spectrum and the standard preparation mass spectrum single point working curve relationship to quantify said analyte comprises a substep of normalizing said first and said standard preparation mass spectrum by dividing each mass spectrum by the respective IRS signals to determine the ratios of analyte signal to IRS signal for each mass spectrum.
- Claim 44. (withdrawn): A method according to claim 35 which involves said working curve analysis using multiple internal reference species, whereby:
- a) said step for ensuring that said specimen contains an IRS further comprises ensuring that said specimen contains a plurality of distinguishable internal reference species each in known and distinct concentrations; and
 - b) said step for capturing and isolating said analyte and said internal reference species using an affinity reagent further comprises capturing and isolating each of said plurality of internal reference species using an affinity reagent; and
 - c) said step for quantifying said analyte using mass spectrometric analysis further comprises: obtaining a mass spectrum of said IRS-containing specimen; whereby said respective mass spectra of said plurality of said internal reference species provide an analyte mass spectrum and a working curve relationship of mass spectra relative to analyte concentration; and then, using said analyte mass spectrum and the internal reference species working curve relationship to quantify said analyte.
- Claim 45. (withdrawn): A method according to claim 44 in which said step for quantifying said analyte further comprises a substep of interpolating or extrapolating the analyte mass spectral signal magnitude relative to at least two internal reference species mass spectral signal magnitudes, the magnitude value of said analyte mass spectral signal corresponding to the quantity of said analyte.
- Claim 46. (withdrawn): A method according to claim 44, in which said step for quantifying said analyte further comprises a substep of estimating the analyte mass spectral signal magnitude relative to at least one internal reference species mass spectral

signal magnitude, the magnitude value of said analyte mass spectral signal corresponding to the quantity of said analyte.

Claim 47. (withdrawn): A method according to claim 44, in which said step for quantifying said analyte further comprises a substep of certifying that the analyte mass spectral signal magnitude is either above or below at least one internal reference species mass spectral signal magnitude, the magnitude value of said analyte mass spectral signal corresponding to the quantity of said analyte.

Claim 48. (currently amended) A method for quantifying a protein in a specimen, said method comprising the steps of:

a) combining said specimen with an internal reference species (IRS) of known concentration, in order to calibrate all subsequent steps; whereby said combination is referred to as an IRS-containing specimen;

b) combining said IRS-containing specimen with an affinity reagent, capturing and isolating said protein and said IRS, wherein said IRS is a modified protein with shifted molecular weight which binds to said affinity reagent; and

c) analyzing and quantifying said protein wherein analyzing and quantifying comprises using ~~only mass spectrometric analysis~~ matrix-assisted laser desorption/ionization (MALDI) to resolve distinct signals for said protein and said IRS to determine the ratio of the protein signal to the IRS signal.

Claim 49. (previously presented) A method according to claim 48, in which said quantifying step further comprises working curve analysis.

Claim 50: (previously presented) A method according to claim 49, in which said working curve analysis comprises substeps of:

a) making a plurality of standard preparations, each containing a known but differing concentration of the protein and each containing a known concentration of IRS;

b) obtaining respective mass spectra of each of the plurality of standard preparations;

- c) normalizing each of the mass spectra from the plurality of standard preparations by dividing each mass spectrum by the IRS signal within the mass spectrum;
- d) creating a working curve by equating the normalized protein signals to the protein concentration of the plurality of standard preparations;
- e) obtaining a mass spectrum for the IRS-containing specimen;
- f) normalizing the mass spectrum of the IRS-containing specimen by dividing by the IRS signal within the mass spectrum; and
- g) quantifying the concentration of the protein in the specimen using the working curve.